REMARKS

Prior to entering the amendment, Claims 1-18 were pending. Claims 7-18 were withdrawn and Claims 1-6 were examined.

The Amendments

In view of the claim amendment, Applicants have amended the first paragraph of the specification to delete the priority claim to U.S. Application No. 09/743,103.

Claim 1 is amended to recite reacting the solubilized cervical sample in the lysis buffer with an antibody against cyclin dependent kinase inhibitor p16. Support for the amendment can be found, for example, in Examples 3 and 4, particularly, at page 24, lines 4-10, and page 27, lines 15-17.

New Claims 19-22 recites the lysis buffer. Support for Claim 19 can be found, for example, at page 8, lines 5-14, and Claim 10 as filed. Support for Claim 20 can be found, for example, at page 23, lines 13-15. Support for Claim 21 can be found, for example, at page 8, lines 30-31, and page 23, lines 13-15. Support for Claim 22 can be found, for example, at page 8, line 6.

New Claims 23 and 24 recite ELISA, and a lateral test flow assay. Support for Claims 23 and 24 can be found, for example, at page 13, lines 9-17.

No new matter is added in any of the above amendments. The Examiner is requested to enter the amendments.

Non-Compliant Amendment

Applicants are re-submitting the corrected version of the non-compliant Amendment to the Specification dated November 15, 2006. The present amendments delete the two paragraphs at page 3, lines 24-28, and add two new paragraph starting at page 3, line 24.

Priority

In view of the claim amendment, Applicants have deleted the priority claim to U.S. Application No. 09/743,103.

35 USC § 102(e) Rejection

9. Claims 1 and 3-6 are rejected under 35 U.S.C. § 102(e), as allegedly being anticipated by U.S. Patent No. 6,709,832 B1, as evidenced by Geradts et al. (*Am. J. Pathol.* 1999 June; 154 (6): 1665-1671). The rejection is overcome in view of the claim amendment.

The '832 Patent broadly teaches a method for detecting cervical carcinomas, cervical intraepithelial neoplasias, or cervical carcinomas in situ, comprising determining the overexpression of cyclin-dependent kinase inhibitor p16 in a human cervical body sample by comparing the expression level of cyclin-dependent kinase inhibitor p16 within said sample to the expression level present in a healthy human cervical body sample. The '832 Patent also illustrates the method by detecting the overexpression of p16 in biopsies of cervix uteri, where the tissue sections were incubated with a p16 antibody and the overexpression was detected by staining the tissue sections (Example 1). However, the '832 Patent does not teach the specific method of the present invention, i.e., solubilizing a cervical body sample in the lysis buffer, and reacting the solubilized cervical sample **in the lysis buffer** with an antibody against cyclin dependent kinase inhibitor p16.

Geradts et al only disclose the Western blot protocol applied on cell monolayers. In the Western blot analysis of Geradts et al, the supernatant of the lysates was first boiled, then the protein was size fractionated by gel electrophoresis, and then transferred to a membrane for detection. The separated protein on the membrane was reacted with an antibody. Generally, the protein/antibody reaction in a Western blot is NOT carried out in the presence of a lysis buffer because the lysis buffer that can solubilize a tissue sample often denatures proteins and inhibits the antigen/antibody binding.

Therefore, the 102(e) rejection of Claims 1 and 3-6 should be withdrawn.

35 USC § 103(a) Rejections

10. Claim 2 is rejected under 35 U.S.C. § 103(a), as allegedly being unpatentable over U.S. Patent No. 6,709,832 B1, as evidenced by Geradts et al. (*Am. J. Pathol.* 1999 June; 154 (6): 1665-1671), in view of Ryder et al. (*Clin. Chem.* 1988 Dec; 34 (12): 2513-2516).

Ryder only discloses procedures of enzyme immunoassays. Ryder does not teach or suggesting reacting a solubilized cervical sample in a lysis buffer with an antibody against cyclin

dependent kinase inhibitor p16, thus it does not cure the deficiency of the '832 Patent.

11. Claims 1 and 3-6 are rejected under 35 U.S.C. § 103(a), as allegedly being unpatentable over Khleif et al. (*Proc. Natl. Acad. Sci. USA.* 1996 Apr; **93**: 4350-4354), as evidenced by Bio-Rad Protein Assay (instruction manual provided with a Bradford assay kit manufactured by Bio-Rad) and the American Type Culture Collection™ (ATCC) catalog, in view of Klaes et al. (*Int. J. Cancer.* 2001; 92: 276-284) (of record; cited by Applicant). The rejection is traversed in parts and overcome in parts in view of the claim amendments.

Khleif et al discloses that p16 is expressed in cervical cancer cell lines in which the RB gene is not functional, either as a consequence of the mutation of RB gene or expression of the human papilloma virus E7 protein. Khleif et al also discloses that p16 levels are increased in primary cells in which RB gene has been inactivated by DNA tumor virus proteins. However, Khleif et al do not disclose that p16 is overexpressed in a cervical carcinoma sample comparing with a healthy human cervical sample.

Khleif et al do not disclose a method for detecting cervical carcinoma, cervical intraepithelial neoplasia, or cervical carcinoma in a human subject. Khleif et al further do not teach a process comprising obtaining a cervical body sample from the human subject to be detected for cervical carcinoma (Claim 1, step a). A cancer cell line disclosed in Khleif is not a cervical body sample from a human to be detected for cervical carcinoma.

Khleif et al disclose the immunoblot analysis of p16 which was present in cervical carcinoma cell lines. Different proteins in the lysate of cervical carcinoma cell lines were separated according to their molecular weight by gel electrophoresis, and electroblotted to a nitrocellulose. The p16 protein blotted on the nitrocellulose was then detected by an anti-p16 antibody in the absence of the lysis buffer. Khleif et al do not teach or suggest reacting the solubilized cervical sample in the lysis buffer with an antibody against cyclin dependent kinase inhibitor p16 (Claim 1, step c).

Klaes et al disclose measuring the overexpression of p16 by immunostaining of the formalin-fixed, paraffin-embedded tissue sections. Klaes et al do not teach or suggest solubilizing a cervical body sample in a lysis sample, let alone reacting a solubilized cervical sample in a lysis buffer with an anti-p16 antibody.

The other secondary references, Bio-Rad Protein Assay and ATCC catalog, do not cure the deficiency of Khleif et al. Therefore, the 103(a) rejection of Claims 1 and 3-6 over Khleif et al, as evidenced by Bio-Rad Protein Assay, and ATCC catalog, in view of Klaes et al should be withdrawn.

12. Claim 2 is rejected under 35 U.S.C. § 103(a), as allegedly being unpatentable over Khleif et al. (*Proc. Natl. Acad. Sci. USA.* 1996 Apr; 93: 4350-4354) in view of Klaes et al. (*Int. J. Cancer.* 2001; 92: 276-284), as applied to claims 1 and 3-6 above, and further in view of Ryder et al. (*Clin. Chem.* 1988 Dec; 34 (12): 2513-2516).

Ryder only discloses procedures of enzyme immunoassays. Ryder does not teach or suggesting reacting a solubilized cervical sample in the lysis buffer with an antibody against cyclin dependent kinase inhibitor p16, thus it does not cure the deficiency of Khleif et al or Klaes et al.

Unexpected Advantages of the Present Invention

The present claims are directed to a method comprising the steps of solubilizing a cervical body sample in a lysis sample, and reacting the solubilized cervical sample in the lysis buffer with an anti-p16 antibody. The steps of the present method are contrary to typical antigen/antibody binding reactions, which are usually carried out in a mild buffer that does not denature proteins. For example, in a typical immunoassay, high concentrations of any detergents are avoided. Anionic detergents (such as SDS) are also avoided in a typical immunoassay. One skilled in the art would not have expected that the p16/anti-p16 binding is NOT inhibited by a lysis buffer that is harsh enough to solubilize a cervical body sample. Prior to the present invention, it was not expected that the immunoreaction between p16 and anti-p16 antibody is not inhibited in the presence of a protein denaturing lysis buffer.

Applicants were the first who discovered that p16 could be measured by an anti-p16 antibody in the presence of a lysis buffer. In view of this discovery, Applicants invented a method for detecting the overexpression of p16 by solubilizing a cervical body sample with a lysis buffer and reacting p16 with an anti-p16 antibody in the presence of the lysis buffer. The present method does not need to separate the p16 protein from other proteins in the lysate, or

remove the lysis buffer before carrying out an immunoreaction. On the contrary, the Western blot method is inconvenient because it involves extra steps of gel electrophoresis and blotting before carrying out an immunoreaction.

In the present application, Applicants have described the unexpected advantages of the invention (page 5, line 24-page 6, line 5), which can provide diagnosis of dysplasias without the cellular morphology information:

> Due to the expression of cyclin dependent kinase inhibitor p16 in certain benign cell types present in cervical specimens, the diagnosis of dysplasias based on the level of cyclin dependent kinase inhibitor p16 without additional information on the cellular morphology seem to be difficult or impossible. It was known in the art that up to 30% of metaplastic cells, which may be present in cervical swabs, are immunoreactive for cyclin dependent kinase inhibitor p16 at a moderate to high level. Moreover, endometrial cells that may under certain circumstances be present in cervical swabs are positive for p16. In cytological or histological testing procedures, this fact does not influence the diagnosis, because the cell types may easily be distinguished from dysplastic cells with respect to their cellular morphology.
> Surprisingly the inventors found that by defining a threshold value of cyclin dependent kinase inhibitor p16, it is possible to enable the detection or diagnosis of dysplasias even without knowledge of the cellular morphology.

Applicants have described the conditions of solubilizing a cervical body sample to preserve the molecular properties of the sample, in which the morphological information of the sample is lost (see Application at page 7, line 24 – page 8, line 22).

Applicants have further reduced the invention to practice. Examples 3 and 4 demonstrate solubilizing human cervical swab samples in a lysis buffer, and determining the overexpression of p16 by reacting with an anti-p16 antibody in the lysis buffer. Such method was not taught or suggested by any of the cited prior art, alone or in combination.

Double Patenting

13. Claims 1 and 3-6 are rejected on the ground of nonstatutory obviousness-type double patenting as allegedly being unpatentable over claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832 in view of Khleif et al., as evidenced by Bio-Rad Protein Assay.

Claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832 do not include steps of solubilizing a cervical body sample in a lysis buffer, and reacting the solubilized cervical sample in the lysis buffer with an antibody against cyclin dependent kinase inhibitor p16. The present claims are not an obvious variation of Claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832 because they specify that the solubilized cervical sample is reacted with the anti-p16 antibody in the lysis buffer. Therefore, the double-patenting rejection of Claims 1 and 3-6 over the '832 Patent should be withdrawn.

14. Claim 2 is rejected on the ground of nonstatutory obviousness-type double patenting, as allegedly being unpatentable over U.S. Patent No. 6,709,832 B1 in view of Khleif et al., as evidenced by Bio-Rad Protein Assay, in further view of Ryder et al.

For the same reasons as stated above, Claim 2 is not an obvious variation of Claims 1, 2, 4, and 5 of U.S. Patent No. 6,709,832. Therefore, the double-patenting rejection of Claim 2 the '832 Patent should be withdrawn.

15. Claims 1 and 3-6 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-26, 33, 34, and 36-40 of copending Application No. 10/633,484 in view of Klaes et al.

Applicants are submitting herewith a Terminal Disclaimer to obviate the provisional double-patenting rejection.

16. As noted in the Office Action, claims 1 and 3-6 are directed to an invention allegedly not patentably distinct from claims 1-26, 33, 34, and 36-40 of commonly assigned copending Application No. 10/633,484.

The subject matter of the '484 Application only qualifies as prior art under 102(e) against the claimed invention. Because at the time this invention was made, the subject matter of the '484 Application and the claimed invention were subject to an obligation of assignment to the same person, i.e., MTM Laboratories AG., the '484 Application shall not preclude patentability of the claimed invention under 35 U.S.C.103(c)(1).

17. Claims 1-6 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-10, 14-16, 42-50, 52-57, and 85 of copending Application No. 10/569,758.

Applicants are submitting a Terminal Disclaimer to obviate the provisional double-patenting rejection.

18. As noted in the Office Action, claims 1-6 are directed to an invention allegedly not patentably distinct from claims of commonly assigned copending Application No. 10/569,758.

Application No. 10/569,758 was filed February 24, 2006, with a PCT filing date of August 20, 2004, which is after the filing date of the present application of August 26, 2003. Therefore, the '758 Application does not qualify as a 103(a) reference.

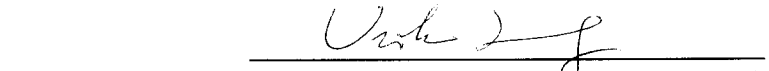
However, as requested by the Examiner, Applicants submit that at the time this invention was made, the subject matter of the '758 Application and the claimed invention were subject to an obligation of assignment to the same person, i.e., MTM Laboratories AG.

CONCLUSION

Applicants believe that the application is now in good and proper condition for allowance. Early notification of allowance is earnestly solicited.

Respectfully submitted,

Date: February 15, 2007



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